

**THIOL METABOLISM IN THE
PARASITIC NEMATODE
*HAEMONCHUS CONTORTUS***

Amanda L. Hudson

PhD

2010



UNIVERSITY OF
TECHNOLOGY SYDNEY



CERTIFICATE OF AUTHORSHIP

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

I also certify that this thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

A handwritten signature in blue ink that reads "Amanda L. Hudson". The signature is written in a cursive style with a large initial 'A'.

Amanda L Hudson

ACKNOWLEDGMENTS

Unquestionably, the person who deserves the biggest thanks is my supervisor Mary Davey. Without you Mary this would not have been possible. Thank you for all your guidance, support, encouragement, friendship and particularly your patience. I know it has not been easy for you having one final student to get rid of, but we did it!

I would also like to give a special thanks to Nick Smith for taking me in when I was officially orphaned and allowing me to become an adopted member of the apicompadres.

To all the students and staff of IBID and MMB, thank you for making it a pleasure to come to work every day. In particular, I would like to thank my fellow 'team meat' members Catherine James and Irene Sotirchos. Without your friendship and guidance I would not have made it through so many years (and still be fairly sane!). Thank you to all my fellow office buddies as well, especially Rowan for putting up with the constant questions, and, Rob, Mike, Alana and Pip for making life entertaining.

I would also like to thank my family and friends, particularly Benjamin Dougall who has had to put up with the 'many moods' associated with being a PhD student.

Special thanks go to John Dalton and Colin Stack for providing the *HcPrx2* plasmid and the antiserum.

Finally I would like to acknowledge the funding given by Meat and Livestock Australia, without which this work would not have been possible, and also the conference funding provided by IBID, the science faculty and the Vice Chancellor.

TABLE OF CONTENTS

CERTIFICATE OF AUTHORSHIP.....	ii
ACKNOWLEDGEMENTS	iii
TABLE OF CONTENTS	iv
ABSTRACT	xi
JOURNAL PUBLICATIONS	xiii
PRESENTATIONS	xiii
LIST OF ABBREVIATIONS	xv
LIST OF FIGURES	xix
LIST OF TABLES	xxiii

Chapter 1: INTRODUCTION	1
1.1. Introduction	2
1.2. <i>H. contortus</i>	2
1.3. Control of <i>H. contortus</i> infections.	4
1.3.1. Vaccine development	5
1.3.2. Drug targets	6
1.4. Resistance to chemotherapy	7
1.5. Antioxidant systems	7
1.6. The glutathione system	11
1.7. The thioredoxin system	13
1.7.1. Thioredoxin	13
1.7.2. Thioredoxin reductase.	14
1.7.3. Peroxiredoxin	17
1.8. Diversity of parasitic antioxidant systems	20
1.8.1. <i>Plasmodium spp.</i>	20

1.8.2. Trypanosomatids	21
1.8.3. Platyhelminths	22
1.8.4. Nematodes	22
1.9. <i>C. elegans</i> as a model.	26
1.10. Thesis objectives	27

Chapter 2: MATERIALS AND METHODS 29

2.1. Materials	30
2.1.1. Chemical reagents	30
2.1.2. Biological reagents	31
2.1.3. Miscellaneous Reagents	32
2.1.4. Bacteria strains	33
2.1.5. <i>Caenorhabditis elegans</i> strains	34
2.1.6. <i>Haemonchus contortus</i> strains	34
2.1.7. Animal strains	34
2.1.8. Cloning vectors	35
2.1.9. Solutions	35
2.2. Methods	37
2.2.1. RNA methods	37
2.2.1.1. mRNA extraction	37
2.2.1.2. TRIzol RNA extraction	37
2.2.1.3. Quantification of nucleic acids	37
2.2.1.4. Determination of RNA quality	38
2.2.1.5. DNase I treatment	38
2.2.1.6. Reverse transcriptase PCR (RT-PCR) using oligo d(T)	38
2.2.1.7. Marathon cDNA amplification	38
2.2.2. DNA methods	38

2.2.2.1.	Touchdown PCR	38
2.2.2.2.	Random amplification cDNA ends (RACE) PCR	39
2.2.2.3.	DNA sequencing	39
2.2.2.4.	DNA sequence analyses	39
2.2.3.	Gene Cloning	40
2.2.3.1.	Nucleic acid electrophoresis	40
2.2.3.2.	DNA gel extraction	40
2.2.3.3.	Restriction digestions.	40
2.2.3.4.	Ethanol precipitation of nucleic acids	40
2.2.3.5.	Ligation reactions	41
2.2.3.6.	Preparation of competent cells	41
2.2.3.7.	Transformation of competent cells	41
2.2.3.8.	Colony PCR	41
2.2.3.9.	Plasmid preparations	42
2.2.3.10.	Glycerol stocks	42
2.2.4.	Real-time quantitative reverse transcriptase (qRT) PCR	42
2.2.4.1.	qRT-PCR reaction conditions	42
2.2.4.2.	Primer Efficiency Analysis: Pfaffl (2001) method . . .	43
2.2.5.	Protein methods	43
2.2.5.1.	Protein extracts	43
2.2.5.2.	Excreted-secreted (ES) fractions	44
2.2.5.3.	Protein sequence analysis	44
2.2.5.4.	Protein Quantification	44
2.2.5.5.	Recombinant protein expression	44
2.2.5.6.	Recombinant protein purification	45
2.2.5.7.	Antiserum Production	45
2.2.5.8.	ELISA	46
2.2.5.9.	Polyacrylamide Gel Electrophoresis	47

2.2.5.10.	Western blotting and detection	47
2.2.6.	Enzyme Assays	47
2.2.6.1.	Peroxiredoxin coupling assay	47
2.2.6.2.	Insulin disulphide reduction kinetic assay	48
2.2.6.3.	Thioredoxin reductase DTNB activity	48
2.2.6.4.	Thioredoxin reductase insulin reduction assay	49
2.2.7.	DNA Nicking assay	49
2.2.8.	<i>C. elegans</i>	50
2.2.8.1.	Solid NGM worm culture	50
2.2.8.2.	Harvesting <i>C. elegans</i> cultures	50
2.2.8.3.	Worm glycerol stocks	50
2.2.8.4.	Synchronous <i>C. elegans</i> cultures	50
2.2.8.5.	<i>C. elegans</i> viability assays	51
2.2.8.6.	Cytotoxic compound exposure	51
2.2.8.7.	MTT assay	51
2.2.8.8.	Worm cytotoxicity assay	52
2.2.9.	RNA interference (RNAi)	52
2.2.9.1.	RNAi controls	52
2.2.9.1.	RNAi	54
2.2.10.	Statistical analyses	55

Chapter 3: THIOREDOXIN REDUCTASES OF *H. CONTORTUS*56

3.1.	Introduction	57
3.2.	<i>H. contortus</i> thioredoxin reductase 2	60
3.3.	Phylogenetic analysis of <i>H. contortus</i> thioredoxin reductases	62
3.4.	Activity of thioredoxin reductase enzymes	66
3.4.1.	Thioredoxin reductase DTNB assay	66

3.4.2. Thioredoxin reductase insulin reduction assay	69
3.4.3. Inhibitor studies	73
3.4.4. DNA nicking assay	76
3.5. Discussion	77

Chapter 4: PEROXIREDOXINS OF *H. CONTORTUS* 86

4.1. Introduction	87
4.2. <i>H. contortus</i> peroxiredoxin 1	87
4.3. <i>H. contortus</i> peroxiredoxin 2	89
4.4. Phylogenetic analysis of <i>H. contortus</i> peroxiredoxins	89
4.5. <i>H. contortus</i> peroxiredoxin assay	93
4.5.1. Enzyme concentrations	96
4.5.2. Peroxiredoxin activity with the thioredoxin system	96
4.5.3. Peroxiredoxin activity with the glutathione system	100
4.5.4. Reduction of hydroperoxides	100
4.5.5. Inhibitor studies	106
4.6. Antioxidant activity	106
4.7. Discussion	111

Chapter 5: *C. ELEGANS* AS A MODEL ORGANISM FOR *H. CONTORTUS* 116

5.1. Introduction	117
5.2. Gene expression of peroxiredoxin and thioredoxin reductase by qRT-PCR	117
5.2.1. Oligonucleotide design	117
5.2.2. Validation of the qRT-PCR assay	119
5.2.3. Expression of antioxidant genes in <i>C. elegans</i>	121
5.3. Gene function	121
5.3.1. Gene KO <i>C. elegans</i>	121

5.3.1.1. Phenotypic changes	123
5.3.1.2. Survival	123
5.3.1.3. Drug sensitivity	123
5.3.2. RNAi in <i>C. elegans</i>	129
5.3.2.1. Probe design	131
5.3.2.2. Knockdown of genes	132
5.4. Discussion	138

Chapter 6: THE FUNCTION OF THE PEROXIREDOXIN AND THIOREDOXIN REDUCTASE ENZYMES IN *H. CONTORTUS*145

6.1. Introduction	146
6.2. Gene expression in <i>H. contortus</i>	146
6.2.1. Oligonucleotide design	146
6.2.2. Validation of the qRT-PCR assay	147
6.2.3. Gene expression in the life cycle stages of <i>H. contortus</i>	148
6.2.4. Gene expression in drug resistant worms	151
6.2.4.1. Gene expression in <i>H. contortus</i> in response to hydrogen peroxide	154
6.3. Native expression and localisation of peroxiredoxin and thioredoxin reductase proteins	157
6.3.1. Antibodies and cross-reactivity to related antioxidants	157
6.3.2. Cellular protein expression in <i>H. contortus</i>	157
6.3.3. Relative protein expression in drug sensitive and drug resistant <i>H. contortus</i> strains	160
6.3.4. Identification of HcPrx2 by <i>H. contortus</i> infected sheep serum	163
6.4. Discussion	163

Chapter 7: CONCLUSIONS AND FUTURE DIRECTIONS171

7.1. Conclusions 172

7.2. Future directions176

REFERENCES179

ABSTRACT

Haemonchus contortus is an important parasitic nematode, both economically and pathologically. The emergence of widespread drug resistance requires new drug or vaccine targets to be identified. The requirement of aerobic organisms to control damage caused by reactive oxygen species and, the increased necessity of parasites to overcome the host immune response, has led to the investigation of antioxidant systems as potential targets. This work examines the thioredoxin antioxidant system in *H. contortus*, specifically the thioredoxin reductase and peroxiredoxin enzymes, to characterise their activity and determine if they are potential targets for parasite control.

H. contortus contains two TrxRs, a cytoplasmic enzyme *HcTrxR1* with a selenocysteine in the active site, similar to the mammalian TrxR, and a mitochondrial enzyme *HcTrxR2* with a nematode unique active site. *HcTrxR1* showed broad activity with thioredoxins from *E. coli*, sheep, and *H. contortus* while *HcTrxR2* had high activity with only the mitochondrial *H. contortus* thioredoxin 1. Importantly, *HcTrxR1* was found to be more sensitive to the black tea inhibitor theaflavin than the selenocysteine containing mammalian TrxR, demonstrating the differences in the enzymes susceptibilities to inhibitors. To determine the function of the TrxR enzymes in nematodes, knockout (KO) strains of *Caenorhabditis elegans* were examined. TrxR1 $-/-$ KO worms were more sensitive to free radical attack and also to the anthelmintic ivermectin; while TrxR2 $-/-$ KO eggs were highly sensitive to sodium hypochlorite. This demonstrates that inhibition of these enzymes would sensitise the nematodes to the host's immune attack.

H. contortus contains two peroxiredoxins, the mitochondrial *HcPrx1* and the cytoplasmic *HcPrx2*. The activity of both peroxiredoxins was specific for the thioredoxin system; however, both peroxiredoxins were also able to be regenerated by the glutathione system when coupled to the nematode specific *H. contortus* thioredoxin 5. Both enzymes were stable to high concentrations of hydrogen peroxide

which demonstrates different functions to their mammalian counterparts. A specific inhibitor of these peroxiredoxins was also identified which has minimal mammalian cytotoxicity. *HcPrx1* was found to be involved in drug resistance while *HcPrx2* was found to be secreted and highly immunogenic. Analysis of homologous genes in *C. elegans* showed that both peroxiredoxin KO worms were sensitive to free radical attack; however, only the cytoplasmic *CePrx2* KO *C. elegans* were sensitive to external oxidants.

Overall, this work adds to the knowledge of *H. contortus* biology and identifies the enzymes of the thioredoxin system as potential drug or vaccine targets for parasite control.

JOURNAL PUBLICATIONS

Hudson, A.L., Sotirchos, I.M. and Davey, M.W. (2010) Substrate specificity of the mitochondrial thioredoxin reductase of the parasitic nematode *Haemonchus contortus*. *Parasitol Res*, *In Press*.

James, C.E., Hudson, A.L. and Davey, M.W. (2009) An update on P-glycoprotein and drug resistance in *Schistosoma mansoni*. *Trends Parasitol*, 25: 538-9.

James, C.E., Hudson, A.L. and Davey, M.W. (2009) Drug resistance mechanisms in helminths: is it survival of the fittest? *Trends Parasitol*, 25:328-35.

Sotirchos, I.M., Hudson, A.L., Ellis, J. and Davey, M.W. (2009) A unique thioredoxin of the parasitic nematode *Haemonchus contortus* with glutaredoxin activity. *Free Radic Biol Med*, 46:579-85.

Sotirchos, I.M., Hudson, A.L., Ellis, J. and Davey, M.W. (2008) Thioredoxins of a parasitic nematode: comparison of the 16- and 12-kDa thioredoxins from *Haemonchus contortus*. *Free Radic Biol Med*, 44: 2026-33.

PRESENTATIONS

Hudson, A.L. and Davey, M.W. (2009) Antioxidants as drug targets for the control of *Haemonchus contortus*, WAAVP, Calgary, Canada.

Hudson, A.L. and Davey, M.W. (2009) A strategy to control parasitic nematodes: The antioxidant system as a drug target, ASP, Sydney, Australia.

Hudson, A.L. and Davey, M.W. (2008) Thiol metabolism and drug resistance in *Haemonchus contortus*, ASP, Adelaide, Australia.

Hudson, A.L., Stack, C., Dalton, J. and Davey, M.W. (2007) Peroxiredoxin, a thiol dependent drug target for *Haemonchus contortus* control, ASP, Canberra, Australia.

Hudson, A.L., Stack, C., Dalton, J. and Davey, M.W. (2007) Peroxiredoxin, a thiol dependent drug target for *Haemonchus contortus* control, MLA research meeting, North Sydney, Australia.

LIST OF ABBREVIATIONS

Abbreviation	Full name
ATP	adenosine triphosphate
BCIP	5-bromo-4-chloro-3-indolyphosphate
BLAST	basic local alignment search tool
Bp	base pair
BSA	bovine serum albumin
°C	degrees Celsius
Cys	cysteine
CDNB	1-chloro-2,4-dinitrobenzene
cDNA	complementary DNA
ddH ₂ O	double-distilled water
DMSO	dimethyl sulfoxide
<i>dpy</i>	dumpy phenotype
dsRNA	double stranded RNA
DNA	deoxyribonucleic acid
dNTPs	deoxyribonucleotide triphosphates
DTNB	5,5-dithiobis(2-nitrobenzoic acid)
DTT	dithiolthreitol
E	efficiency
EDTA	ethylene diamine tetra acetic acid
ELISA	enzyme-linked immunosorbent assay
ES	excretory-secretory
EtOH	ethanol
FA	Formaldehyde agarose
FAD	flavin adenine dinucleotide
<i>g</i>	centrifugal force (gravity)
G	gauge

GR	glutathione reductase
Grx	glutaredoxin
GSH	reduced glutathione
GSSG	oxidised glutathione
GPx	glutathione peroxidase
Hrs	hour
IC ₅₀	inhibitory concentration (50%)
Ig	immunoglobulin
IPTG	isopropyl- β -thiogalactopyranoside
IVF	ivermectin resistant <i>H. contortus</i> strain
IVM	ivermectin
Kb	kilobases
K _{cat}	catalytic constant
kDa/K	kilodaltons
K _i	inhibitory constant
K _m	Michaelis constant
KO	knockout
L1	first larval stage
L2	second larval stage
L3	third larval stage
L4	fourth larval stage
LB	Luria broth base
<i>Lon</i>	Longer phenotype
m	metres
M	molar
MES	2-(N-morpholino) ethanesulfonic acid
MFO	mixed function oxidase
mins	minutes

MOF	moxidectin resistant <i>H. contortus</i> strain
MOPS	3-(N-morpholino) propanesulfonic acid
M _r	molecular weight
mRNA	messenger RNA
MSG	monosodium glutamate
MTT	3-(4,5 dimethylthiazolyl-2)-2,5-diphenyl tetrazolium
MWCO	molecular weight cut off
NADH	beta-nicotinamide adenine dinucleotide
NADPH	beta-nicotinamide adenine dinucleotide phosphate reduced form tetrasodium
NBT	nitro blue tetrazolium
ng	nanograms
NGM	nematode growth media
nm	nanometre
OD	optical density
ORF	open reading frame
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PDI	protein disulfide isomerase
PFC	4-phenyl-3-furoxan carbonitrile
pI	isoelectric point
PMSF	phenyl methane sulphonyl fluoride
Prx	peroxiredoxin
qRT-PCR	quantitative reverse transcriptase PCR
QS	Quackenbush
RACE PCR	random amplification cDNA polymerase chain reaction
RNA	ribonucleic acid

RNAi	RNA interference
ROS	reactive oxygen species
rpm	revolutions per minute
RT	room temperature
RT-PCR	reverse transcriptase polymerase chain reaction
SECIS	selenocysteine insertion sequence
secs	seconds
SeCys/U	selenocysteine
SDS	sodium dodecyl sulphate
siRNA	smaller interfering RNAs
SOD	superoxide dismutase
TBE	Tris borate buffer with EDTA
TF	theaflavin
TFBI/II	transformation buffer I/II
TGFβ	transforming growth factor beta
TGR	thioredoxin glutathione reductase
T _m	melting temperature
TNB	2-nitro-5-thiobenzoate
Trx	thioredoxin
TrxR	thioredoxin reductase
Try/TXN	trypanothione
TryR	trypanothione reductase
UV	ultraviolet
V	Volts

LIST OF FIGURES

Chapter 1: INTRODUCTION

Figure 1.1: Life cycle of <i>Haemonchus contortus</i>	3
Figure 1.2: First line of defence against reactive oxygen species	10
Figure 1.3: The glutathione and thioredoxin systems	12
Figure 1.4: Schematic of thioredoxin reductase and related enzymes	15
Figure 1.5: Schematic of peroxiredoxin enzymes	19
Figure 1.6: Schematic representation of <i>H. contortus</i> thioredoxins	24

Chapter 2: MATERIALS AND METHODS

Figure 2.1: BLASTN taxonomy report for pcb19 negative control RNAi probe	53
--	----

Chapter 3: THIOREDOXIN REDUCTASES OF *H. CONTORTUS*

Figure 3.1: Amino acid alignment of the HCC04397 contig with <i>C. elegans</i> TrxR1 .	58
Figure 3.2: Expression of <i>HcTrxR1</i>	59
Figure 3.3: Amino acid alignment of the CB064883 EST with <i>C. elegans</i> TrxR2 and <i>C. elegans</i> GR	61
Figure 3.4: Cloning and expression of <i>HcTrxR2</i>	63
Figure 3.5: Alignment of thioredoxin reductase enzymes from <i>H. contortus</i> and <i>C. elegans</i>	64
Figure 3.6: Neighbour-joining phylogenetic tree of thioredoxin reductase-like oxidoreductase protein sequences from 11 different species	65
Figure 3.7: Multiple sequence alignment of thioredoxin reductases	68
Figure 3.8: Velocity of TrxR enzymes using the substrate DTNB (A) and <i>HcTrx1</i> (B)	70
Figure 3.9: Activity of TrxR enzymes measuring the reduction of thioredoxins . . .	72
Figure 3.10: Activity of recombinant <i>H. contortus</i> TrxR enzymes with different substrates	74
Figure 3.11: Activity of TrxR enzymes in the presence of known mammalian inhibitors	75
Figure 3.12: Antioxidant protection of plasmid DNA by <i>HcTrxR1</i> and <i>HcTrxR2</i>	78

Figure 3.13: Selenocysteine insertion (SECIS) element of <i>HcTrxR1</i>	80
---	----

Chapter 4: PEROXIREDOXINS OF *H. CONTORTUS*

Figure 4.1: Amino acid alignment of the HCC04714 Contig with <i>C. elegans</i> Prx1 . .	88
Figure 4.2: Cloning and expression of <i>HcPrx1</i>	90
Figure 4.3: Cloning and expression of <i>HcPrx2</i>	91
Figure 4.4: Amino acid alignment of peroxiredoxin proteins from <i>H. contortus</i> and <i>C. elegans</i>	92
Figure 4.5: Neighbour-joining phylogenetic tree of typical 2-Cys peroxiredoxins from different species	94
Figure 4.6: Effect of thioredoxin (<i>HcTrx1</i>) and thioredoxin reductase (rat TrxR) concentrations on peroxiredoxin activity	97
Figure 4.7: Effect of NADPH concentrations on peroxiredoxin activity	98
Figure 4.8: Activity of <i>HcPrx1</i> and <i>HcPrx2</i> using different thioredoxins	99
Figure 4.9: Activity of <i>H. contortus</i> peroxiredoxin enzymes with thioredoxin reductase enzymes	101
Figure 4.10: Activity of <i>HcPrx1</i> and <i>HcPrx2</i> coupled with the glutathione (GSH) system or the GSH system with the addition of <i>HcTrx5</i>	102
Figure 4.11: Effect of hydrogen peroxide concentration on peroxiredoxin activity	104
Figure 4.12: The ability of <i>HcPrx1</i> and <i>HcPrx2</i> to reduce other substrates	105
Figure 4.13: Activity of <i>H. contortus</i> peroxiredoxin enzymes with the catalase inhibitor aminotriazole	107
Figure 4.14: Inhibition of peroxiredoxin activity in the presence of 4-Phenyl-3-furoxancarbonitrile (PFC)	108
Figure 4.15: Effect of 4-phenyl-3-furoxancarbonitrile (PFC) on the activity of <i>HcTrx1</i> and rat TrxR	109
Figure 4.16: Antioxidant activity of <i>HcPrx1</i> and <i>HcPrx2</i>	110

Chapter 5: *C. ELEGANS* AS A MODEL ORGANISM FOR *H. CONTORTUS*

Figure 5.1: Linear regression of CT versus template concentration of <i>C. elegans</i> in qRT-PCR reactions	120
---	-----

Figure 5.2: Expression of antioxidant genes in <i>C. elegans</i> worms treated with cytotoxic compounds	122
Figure 5.3: Comparison of adult <i>C. elegans</i> worms	124
Figure 5.4: Survival of <i>C. elegans</i> after alkaline hypochlorite treatment	125
Figure 5.5: Linearity of formazan production in L1 larvae of <i>C. elegans</i>	126
Figure 5.6: Viability of <i>C. elegans</i> following paraquat treatment	127
Figure 5.7: Viability of <i>C. elegans</i> following hydrogen peroxide treatment	128
Figure 5.8: Viability of <i>C. elegans</i> following ivermectin treatment	130
Figure 5.9: RNAi of peroxiredoxin genes in wild type Bristol N2 <i>C. elegans</i>	134
Figure 5.10: RNAi of the thioredoxin reductase in wild type Bristol N2 <i>C. elegans</i> .	135
Figure 5.11: RNAi of peroxiredoxin genes in Bristol N2 <i>C. elegans</i> on 0.1 M paraquat NGM plates	136

Chapter 6: THE FUNCTION OF THE PEROXIREDOXIN AND THIOREDOXIN REDCUTASE ENZYMES IN *H. CONTORTUS*

Figure 6.1: Linear regression of CT versus template concentration of <i>H. contortus</i> qRT-PCR reactions	149
Figure 6.2: Expression of the peroxiredoxin and thioredoxin reductase genes in the life cycle of <i>H. contortus</i>	150
Figure 6.3: Expression of antioxidant genes in drug resistant <i>H. contortus</i> strains .	152
Figure 6.4: Expression of antioxidant genes in <i>H. contortus</i> strains from different sheep	153
Figure 6.5: Expression of antioxidant genes in drug resistant <i>H. contortus</i> strains .	155
Figure 6.6: Response of <i>HcPrx1</i> , <i>HcPrx2</i> , <i>HcTrxR1</i> and <i>HcTrxR2</i> genes to hydrogen peroxide treatment in drug sensitive (Kirby1981) and multidrug resistant (Wallangra2003) <i>H. contortus</i> strains	156
Figure 6.7: Cross-reactivity using antisera with recombinant <i>H.contortus</i> proteins	158
Figure 6.8: Linearity of polyclonal antibodies against recombinant proteins	159
Figure 6.9: Identification of <i>HcPrx1</i> and <i>HcPrx2</i> in <i>H. contortus</i> extracts	161
Figure 6.10: Identification of <i>HcTrxR1</i> and <i>HcTrxR2</i> in <i>H. contortus</i> extracts	162

Figure 6.11: Band intensities of *HcPrx1*, *HcPrx2* and *HcTrxR2* identified in *H. contortus* total protein extracts 164

Figure 6.12: Identification of *HcPrx2* in the excretory-secretory (ES) fraction of *H. contortus* 165

Figure 6.13: Identification of *HcPrx2* by *H. contortus* infected sheep serum166

LIST OF TABLES

Chapter 1: INTRODUCTION

Table 1.1: Antioxidant enzymes associated with drug resistance in parasites (James *et al.*, 2009a)8

Table 1.2: C-terminal active sites of TrxR enzymes from different organism17

Chapter 3: THIOREDOXIN REDUCTASES OF *H. CONTORTUS*

Table 3.1: Percentage similarities of thioredoxin reductase-like oxidoreductase protein sequences from different species 67

Table 3.2: Kinetic constants for the TrxRs with *HcTrx1* 71

Table 3.3: Ki values for inhibitors of thioredoxin reductase enzymes 76

Chapter 4: PEROXIREDOXINS OF *H. CONTORTUS*

Table 4.1: Percentage similarities of peroxiredoxin protein sequences from different species 95

Table 4.2: Kinetic constants for the *H. contortus* peroxiredoxin enzymes using different substrates103

Chapter 5: *C. ELEGANS* AS A MODEL ORGANISM FOR *H. CONTORTUS*

Table 5.1: Primers for quantitative real-time PCR for *C. elegans* 118

Table 5.2: *C. elegans* qRT-PCR primer efficiencies 119

Table 5.3: Specificity of RNAi probes determined using E-RNAi 131

Table 5.4: Primers for the amplification of peroxiredoxin and thioredoxin reductase RNAi probes from cDNA 132

Table 5.5: Relative fold change in gene expression levels in RNAi treated worms determined by qRT-PCR 137

Table 5.6: Sensitivity of gene KO *C. elegans* to cytotoxic compounds 141

Table 5.7: Summary of RNAi results143

Chapter 6: THE FUNCTION OF THE PEROXIREDOXIN AND THIOREDOXIN REDCUTASE ENZYMES IN *H. CONTORTUS*

Table 6.1: Primers for quantitative real-time PCR for *H. contortus* 147

Table 6.2: *H. contortus* qRT-PCR primer efficiencies 148